

We claim:

1. A method of using immortalized human hepatocyte cells to produce a protein comprising the steps of:

5 (a) providing an immortalized human hepatocyte cell that includes DNA that encodes and can express a protein;

(b) culturing the immortalized hepatocyte cell under conditions in which a gene or genes encoding the protein are expressed so that the protein is produced and processed in the immortalized hepatocyte cell; and

10 (c) isolating the processed protein from the immortalized hepatocyte cell; wherein the protein is expressed such that the protein is processed and glycosylated, if necessary, so that its *in vivo* function is substantially preserved after its isolation.

15 2. The method of claim 1 wherein the protein is a plasma protein that is naturally produced by human hepatocytes.

3. The method of claim 1 wherein the protein is a protein that is not naturally produced by human hepatocytes.

20 4. The method of claim 3 wherein the protein is a mutein of a protein that is normally produced by human hepatocytes.

5. The method of claim 1 wherein the protein is a therapeutic protein.

25 6. The method of claim 5 wherein the therapeutic protein is a therapeutic plasma protein.

7. The method of claim 6 wherein the protein is selected from the group consisting of Factor VIII, Factor IX, human growth hormone (hGH),  $\alpha$ -1-antitrypsin, and a growth factor.

30

8. The method of claim 6 wherein the protein is selected from the group consisting of muteins of Factor VIII, muteins of Factor IX, muteins of human growth hormone, muteins of  $\alpha$ -1-antitrypsin, and muteins of a growth factor.

5

9. The method of claim 1 wherein the protein is an I $\alpha$ Ip protein complex.

10. The method of claim 1 wherein the protein is a protein selected from the group consisting of albumin, transcobalamin II, C-reactive protein, fibronectin,  
10 ceruloplasmin, and other proteins having structural, enzymatic, or transport activities.

11. The method of claim 1 wherein the protein is a mutein of a protein selected from the group consisting of albumin, transcobalamin II, C-reactive protein, fibronectin, ceruloplasmin, and other proteins having structural, enzymatic, or transport activities.

15

12. The method of claim 1 wherein the protein is expressed by a gene that occurs naturally in the hepatocytes, and expression of the naturally-occurring gene encoding the protein is enhanced by introduction of a high-level promoter into the hepatocytes.

20

13. The method of claim 1 wherein expression is enhanced by introducing multiple copies of the gene encoding the protein to be expressed, a subunit of the protein to be expressed, or a precursor of the protein to be expressed via the use of one or more recombinant vectors that include: (1) the gene encoding the protein to be expressed, a  
25 subunit of the protein to be expressed, or a precursor of the protein to be expressed; and (2) at least one control element affecting the transcription of the gene, the control element being operably linked to the gene.

14. The method of claim 13 wherein the recombinant vector is selected from  
30 the group consisting of SV40-derived vectors, murine polyoma-derived vectors, BK virus-

derived vectors, Epstein-Barr virus-derived vectors, adenovirus-derived vectors, adeno-associated virus-derived vectors, baculovirus-derived vectors, herpesvirus-derived vectors, lentiviral-derived vectors, retrovirus-derived vectors, alphavirus-derived vectors, and vaccinia virus-derived vectors.

5

15. The method of claim 14 wherein the vector incorporates one or more reporter genes.

10 16. The method of claim 1 wherein the expressed protein is secreted from the cell into the surrounding culture medium.

17. The method of claim 1 wherein the protein is glycosylated.

15 18. The method of claim 1 wherein the protein is processed post-translationally.

19. The method of claim 1 wherein the protein is expressed in a form wherein it is fused to a cleavable tag.

20 20. The method of claim 19 wherein the cleavable tag is selected from the group consisting of glutathione *S*-transferase, the MalE maltose-binding protein, and a polyhistidine sequence.

25 21. The method of claim 1 wherein the protein comprises at least two different subunits, and wherein the immortalized hepatocyte cell is transformed or transfected with at least two vectors, each vector including: (1) DNA including at least one gene that encodes at least one subunit of the protein; and at least one control element operably linked to the DNA encoding at least one gene that encodes the subunit of the protein.

30 22. The method of claim 1 wherein the immortalized human hepatocyte cell is virally immortalized.

23. The method of claim 22 wherein the hepatocyte is immortalized by transformation or transfection with substantially pure simian virus (SV40) DNA.

5 24. The method of claim 23 wherein the substantially pure SV40 DNA encodes large T and small t antigens (Tag).

25. The method of claim 1 wherein the immortalized human hepatocyte cell is derived from primary cryopreserved human hepatocytes.

10 26. The method of claim 1 wherein the hepatocyte includes tumor-suppressor-encoding DNA such that substantially pure DNA encoding tumor suppressor can be isolated and purified from the hepatocyte.

15 27. The method of claim 1 wherein the hepatocyte includes DNA encoding Rb such that substantially pure DNA encoding Rb can be isolated and purified from the hepatocyte.

20 28. The method of claim 1 wherein the hepatocyte includes DNA encoding p53 such that substantially pure DNA encoding p53 can be isolated and purified from the hepatocyte.

25 29. The method of claim 1 wherein the hepatocyte is nontumorigenic, has the ability to be maintained in a serum-free medium, and produces plasma proteins.

30 30. The method of claim 1 wherein the hepatocyte is a hepatocyte of the Fa2N-4 cell line.

35 31. The method of claim 1 wherein the hepatocyte is a hepatocyte of the Ea1C-35 cell line.

32. A method of using eukaryotic cells, other than human hepatocytes, to produce an I $\alpha$ Ip protein complex comprising the steps of:

(a) providing a eukaryotic cell, other than a human hepatocyte, that includes DNA that encodes and can express proteins forming an I $\alpha$ Ip protein complex, the eukaryotic cell having been transformed or transfected with at least one vector that includes: (1) DNA including at least one gene for a precursor of a protein that is part of an I $\alpha$ Ip protein complex; and (2) at least one control element operably linked to the DNA encoding at least one precursor gene in order to enhance expression of the precursor gene;

(b) culturing the transformed or transfected eukaryotic cell under conditions in which genes encoding proteins forming an I $\alpha$ Ip complex are expressed so that an I $\alpha$ Ip complex is produced; and

(c) isolating the expressed I $\alpha$ Ip protein complex from the transformed or transfected eukaryotic cell.

33. The method of claim 32 wherein the eukaryotic cell is selected from the group consisting of CHO cells, COS cells, and yeast cells.

34. The method of claim 32 wherein the hepatocyte is transformed or transfected with two vectors: (1) a first vector that includes the genes *H3* and *AMB*P; and (2) a second vector that includes the genes *H2* and *H1*.

35. An immortalized human hepatocyte cell that includes DNA that encodes and can express a protein, the immortalized human hepatocyte cell having been transformed or transfected with at least one vector that includes: (1) DNA including at least one gene encoding a protein; and (2) at least one control element operably linked to the DNA encoding the protein in order to enhance expression of the protein.

36. The cell of claim 35 wherein the protein is a protein that is naturally produced by human hepatocytes.

37. The cell of claim 35 wherein the protein is a protein that is not naturally produced by human hepatocytes.

5 38. The cell of claim 37 wherein the protein is a mutein of a protein that is normally produced by human hepatocytes.

39. The cell of claim 35 wherein the protein is a therapeutic protein.

10 40. The cell of claim 39 wherein the therapeutic protein is a therapeutic plasma protein.

41. The cell of claim 40 wherein the protein is a therapeutic plasma protein selected from the group consisting of Factor VIII, Factor IX, human growth hormone (hGH),  $\alpha$ -1-antitrypsin, and a growth factor.

15 42. The cell of claim 40 wherein the protein is a plasma protein is selected from the group consisting of muteins of Factor VIII, muteins of Factor IX, muteins of human growth hormone, muteins of  $\alpha$ -1-antitrypsin, and muteins of a growth factor.

20 43. The cell of claim 35 wherein the plasma protein is an I $\alpha$ Ip protein complex.

44. The cell of claim 35 wherein the protein is a protein selected from the group consisting of albumin, transcobalamin II, C-reactive protein, fibronectin, ceruloplasmin, and other proteins having structural, enzymatic, or transport activities.

25 45. The cell of claim 45 wherein the protein is a mutein of a protein selected from the group consisting of albumin, transcobalamin II, C-reactive protein, fibronectin, ceruloplasmin, and other proteins having structural, enzymatic, or transport activities.

46. A method of treating a disease or condition comprising the steps of:  
(a) providing an active protein produced according to the method of claim 1; and  
(b) administering the active protein to a patient suffering from the disease or condition in a therapeutically active quantity to treat the disease or condition.

5

47. The method of claim 46 wherein the disease or condition is a disease or condition affecting the liver.

48. The method of claim 47 wherein the disease or condition affecting the liver is selected from the group consisting of sepsis, cancer, hepatitis, and liver failure.

10

49. The method of claim 46 wherein the disease or condition is a disease or condition affecting an organ other than the liver.

15

50. The method of claim 49 wherein the disease or condition is selected from the group consisting of cancer, joint inflammation, and arthritis.

51. A pharmaceutical composition for treating a disease or condition comprising:

20

(a) an I $\alpha$ I $\beta$  protein complex produced by eukaryotic cells in a quantity therapeutically effective to treat a disease or condition; and  
(b) a pharmaceutically acceptable carrier.

25

52. The pharmaceutical composition of claim 51 wherein the disease or condition is a disease or condition affecting the liver.

53. The pharmaceutical composition of claim 52 wherein the disease or condition affecting the liver is selected from the group consisting of sepsis, cancer, hepatitis, and liver failure.

30

54. The pharmaceutical composition of claim 51 wherein the disease or condition is a disease or condition affecting an organ other than the liver.

55. The pharmaceutical composition of claim 54 wherein the disease or condition is selected from the group consisting of cancer, joint inflammation, and arthritis.

56. A method of treating a disease or condition comprising the steps of:  
(a) providing an active plasma protein produced by the method of claim 1; and  
(b) administering the active plasma protein to a patient suffering from the disease or condition in a therapeutically effective quantity to treat the disease or condition.

57. The method of claim 56 wherein the disease or condition is a disease or condition affecting the liver.

58. The method of claim 57 wherein the disease or condition affecting the liver is selected from the group consisting of sepsis, cancer, hepatitis, and liver failure.

59. The method of claim 56 wherein the disease or condition is a disease or condition affecting an organ other than the liver.

60. The method of claim 59 wherein the disease or condition is selected from the group consisting of cancer, joint inflammation, and arthritis.

61. The method of claim 56 wherein the active plasma protein is selected from the group consisting of Factor VIII, Factor IX, human growth hormone (hGH),  $\alpha$ -1-antitrypsin, and a growth factor.